BOTULINUM TOXIN INJECTION OF EYE MUSCLES TO CORRECT STRABISMUS*

BY *Alan B. Scott,* MD

INTRODUCTION

THE CORRECTION OF NON-ACCOMMODATIVE STRABISMUS REQUIRES SURGICAL MAnipulation of the extraocular muscles to alter alignment of the eyes. The present study was designed to develop and evaluate an alternative treatment for strabismus; injection of drugs into the muscle to weaken it, rather than surgical manipulation to do so. According to Knapp,[1] Dr Conrad Berens injected alcohol into extraocular muscles for this purpose; however, the effects were either inadequate, or occasionally a total and permanent paralysis, and the experiments were abandoned. Bach-y-Rita,[2] Crone,[3] Irvine,[4] Jampolsky,[5] and Villa-Seca[6] also had the notion of drug injection or did it with local anesthetics, but experiments were not pursued or published. We describe below our experience with botulinum A toxin, its characteristics, preparation, and the results in monkeys and humans from injection of botulinum A toxin into extraocular muscles as a therapeutic modality. Preliminary animal studies[7] and preliminary studies in a few patients are already published.[8,9]

BOTULINUM TOXIN

Botulinum toxin is produced by *Clostridium botulinum* and includes six antigenically distinguishable toxins, labeled A, B, C, D, E, and F. More work has been done with type A than with any of the other toxins, in part because most strains of this type retain their toxigenicity well, yield highly potent culture fluids (10^6 mouse LD/ml) in a variety of media, and the toxin can easily be crystallized in a stable form.[10,11] Only type A was used in this investigation. Type A appears to have great muscle paralytic effect in humans.

*Supported by NIH Grant No. 1 R01 EY02106 and The Smith-Kettlewell Eye Research Foundation.

PHYSICAL PROPERTIES

Considerable work has been done in efforts to determine the properties of the type A toxin molecule. The "crystalline toxin" stable in acid solutions was found to have a molecular weight of 900,000 daltons, and ultraviolet absorption maximum at 278 nm.[12-14] However, it was subsequently demonstrated[15,16] that the neuro-toxic activity and the hemagglutinating activity could be separated by adsorption of the hemagglutinin on red blood cells, and that the toxin and the hemagglutinin dissociated spontaneously in a neutral or mildly alkaline solution. Recent reports[17-21] are in general agreement on molecular weight for type A neuro-toxin of 140,000 to 150,000 daltons, and radius of 4.8 nm with the heavier hemagglutinin fraction being found in three states of aggregation—molecular weights of 290,000; 500,000; and 900,000 daltons. The radius has been confirmed by electron microscopy[22] which showed the toxin to be disc-like particles of 4 to 4.5 nm, agglomerating to form long double strands. The pure toxin of molecular weight 150,000 is three to five times as toxic as the crystalline toxin-hemagglutinin.[17,23]

CHEMICAL COMPOSITION

The chemical composition of botulinum toxin can be completely accounted for by amino acid analyses[20,24] (Table I). It was postulated that tryptophane residues in type A botulinum toxin were either located in or were contributing to the formation of reactive sites involved in the mainte-

TABLE I: ORDER OF RAPIDITY OF DEATH OF RABBITS BY VARIOUS ROUTES OF INJECTION OF A GIVEN QUANTITY OF BOTULINUM TOXIN (34) (SYMPTOMS OF POISONING WERE SIMILAR BY THE VARIOUS ROUTES OF INJECTION)

ROUTE	TIME OF DEATH
Intravenous	2 hours 25 minutes
Intra-arterial	2 hours 35 minutes
Intramuscular	4 hours 30 minutes
Intracerebral	4 hours 35 minutes
Intrapulmonary	5 hours
Occipital	6 hours 10 minutes
Subcutaneous	7 hours 45 minutes
Eye anterior chamber	8 hours 15 minutes
Intraperitoneal	9 hours 5 minutes
Intrasciatique	10 hours 53 minutes
Intragastric	33 hours 45 minutes
Intrarectal	4 days 9 hours

nance of toxicity as well as in the formation of neutralizing and protective antibodies.[25] This view has been challenged by others[26] who suggested that cysteine is more likely to be of importance in the reactive sites of the toxin. At present, it seems probable that a number of amino acids at various locations in the peptide chain are a part of the reactive site. "The toxin appears to be single polypeptide chain without prosthetic groups whose maintenance of integrity of tertiary structure (three dimensional relationships) appears necessary for toxicity."[4]

ABSORPTION AND UPTAKE

The toxin-hemagglutinin complex (with molecular weight of 900,000 daltons) would appear to be too large to be able to penetrate through the intestinal wall of orally intoxicated animals; it is, in fact, found in their blood circulation as 150,000 daltons.

There is little evidence for the ability of the toxin to penetrate the blood-brain barrier and act centrally.[27] Demonstrations of localization of the toxin in the brain histologically using labeled toxin have been inconclusive because the toxin preparations used were only partially purified; perhaps the radio-label detected in brain tissue was bound to some contaminants rather than to the toxin.[28] Other studies with an impure toxin[29] have failed to reveal any localization in the brain tissue. A recent study did use a radioiodinated preparation of pure toxin.[30] Mice were injected with massive doses (150,000 MLD) which usually caused death within 35 minutes. Examination of brain tissue indicated the presence of some radio-labeled substance in the blood vessels and in the parenchyma of the brain. It would seem that only in massive doses is there any toxin remaining in circulation to pass through the blood-brain barrier.

Dawson and Simpson[31] implanted electrodes in the optic nerve and visual cortex of rabbits who were then injected intravenously with highly potent doses of toxin. No change in electrophysiologic function of the visual pathway was observed during the six to ten hours preceding death.

Botulinum toxin binds very rapidly and quite firmly to muscle[27,32] and if injected locally into a given muscle group, it will continue to act for a prolonged period. In very low doses then, injected specifically into an eye muscle, the available toxin should be rapidly and firmly bound to that muscle, leaving little toxin to pass into the circulatory system to cause systemic effects.

For other botulinum types, toxin has been detectable in the blood up to 25 days after oral ingestion. Attempts to demonstrate this with type A toxin have been generally unsuccessful due to more rapid removal of type A toxin from the circulation and its fixation at terminal nerve fibers and

other tissue binding loci. There is apparently only one case (which was fatal) in which type A toxin could be isolated from a blood sample.[33]

DOSE-RESPONSE

Sensitivity seems to be related strongly to route of administration because of differences in penetrability of epithelial tissues by the drug and in extent of surface area of tissue available for absorption or penetration[34] (Table I).

For a *single* route of administration, however, Boroff and Fleck[35] established that within a relatively wide range of concentrations, the survival time of mice injected intravenously was in a linear relationship with the reciprocal of the log of concentration of the toxin. This has become the basis of toxicity testing by mouse LD/50 injection in the experiments reported here.

For humans, there are widely different estimates of the amount of toxin required to induce the disease of botulism or to cause death. One estimate of the parenteral lethal dose for a fully grown man was seven mouse lethal doses[10]; however, we have given 26 mouse LD/50 doses as an eye muscle injection without any systemic effect. An estimate of 2.0 μg is the human LD/50 which might be supposed by reference to our experience in monkeys injected into the orbit. Following oral ingestion, the incubation period in man has been observed to be extremely variable, ranging from 18 hours to several days.[36] As in clinical intoxication, we find larger doses create earlier and a more profound effect upon extraocular muscle injection.

MECHANISM OF ACTION

Botulinum toxin interferes with the acetylcholine release from the nerve terminal rather than interfering with the storage of acetylcholine in vesicles.[37] It appears that the toxin acts upon individual motor nerve terminals, because not all muscle fibers belonging to a motor unit fail at one time.[38,39] The action is gradual and continues until the end plate potential is diminished to an irreducible quantity, occasional miniature end plate potentials associated with release of each quanta of acetylcholine.

Boroff and Das Gupta[25] suggested that "botulinum toxin in some ways antagonizes calcium ion transport by serotonin. After depletion of calcium ions, the end plate does not release acetylcholine and the muscle fiber fails to contract." This hypothesis is supported by Thesleff's finding[40] that the end plate potentials could be temporarily restored by doubling the calcium ion concentration bathing the intoxicated nerve muscle preparation. Work on botulinum-poisoned skeletal muscle (in vivo) also indicates

that the transmitter release mechanism is intact but that it requires a higher than usual level of calcium. By the use of guanidine, or 4-amino pyridine, an approximately normal level of transmitter release occurred; this may explain the basis for improvement of marked fatigability, ptosis, extraocular palsies, and evoked action potentials in cases of human botulism following guanidine treatment.[41-44]

Botulinum toxin does not block propagation of the nerve impulse; neither nerve nor muscle suffers impairment of electrical excitability or conductivity. A post-mortem examination of human neuromuscular apparatus following death from botulism demonstrated "anatomically normal nerves, nerve terminals, end-plate, and muscles. Both nerve conduction and the ability of muscles that were paralyzed the fifth day preceding death to contract to direct electrical stimulation were normal.[45]"

Long term exposure to the toxin does invariably cause atrophy of the muscle. The changes in botulinum treated skeletal muscles are consistent in kind and in degree with the effects of denervation.[27] It has been demonstrated that cardiac muscle remains functionally and morphologically intact even after prolonged treatment with the toxin in the chick embryo.[46] Although cardiac muscle is striated (as are the skeletal muscles), it is not dependent on its innervation for maintaining its integrity. The incidence and frequency of signs and symptoms reported in cases of type A botulism in the United States between 1953 and 1973 are listed in Table II. The appearance or presence of these signs and symptoms is consistent with the accepted action of botulinum toxin as a presynaptic blocking agent for cholinergic junctions and the neuromuscular block of efferent fibers to the skeletal muscles (which would account for the general weakness).

ACTION ON THE EYE

The first neurological disturbances observed after orally ingested toxin in many cases are disturbances of vision due to paralysis of intraocular and extraocular muscles from pupillary dilation, impairment of accommodation, ptosis, and diplopia. Complete recovery from palsies of intraocular and extraocular muscles may require as long as six to nine months.[47] In mild cases, recovery was judged complete within two to three months.[43] This mechanism of prolonged paralysis and atrophy of the injected muscle over a sufficient time to allow antagonist contracture is ideal for our purpose in strabismus treatment.

TABLE II: SYMPTOMS AND SIGNS REPORTED BY ONE OR MORE PERSONS DURING OUTBREAKS OF BOTULISM TYPE A, 1953 TO 1973 (11)

SYMPTOMS	% REPORTED DURING OUTBREAKS
Blurred vision, diplopia, photophobia	91
Dysphagia	79
Dysphonia	74
Generalized weakness	65
Nausea, vomiting	44
Dizziness or vertigo	24
Abdominal pain, cramps, fullness	15
Diarrhea	15
Sore throat	12
Urinary retention or incontinence	6
Constipation	0*
SIGNS	
Respiratory impairment	94
Specific muscle weakness or paralysis	68
Eye muscle weakness, including ptosis	47
Dry throat, mouth, tongue	21
Dilated, fixed pupils	9
Ataxia	9
Nystagmus	3
Postural hypotension	0*
Somnolence	0*

*Reported during outbreaks of botulism other than type A.

ANIMAL EXPERIMENTS

ALIGNMENT CHANGES

Thirteen horizontal rectus muscles of eight rhesus monkeys were injected with this toxin.[7] The amount injected varied between 1×10^{-5} μg and 1.6×10^{-3} μg, in volumes between 5 μl and 500 μl. We were able to produce both transient weakness of individual horizontal muscles, varying between two weeks and eight months, and permanent changes of ocular alignment, depending upon the concentration of drug injection. Diffusion of drug to adjacent extraocular or levator muscles occurred in higher doses; systemic effect was never observed. Figure 1 demonstrates

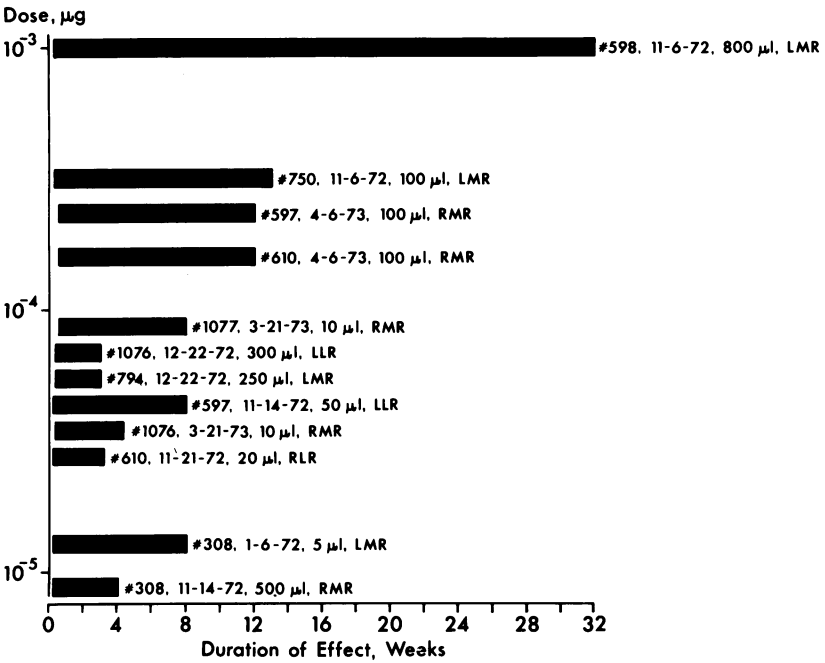

FIGURE 1
The duration of induced strabismus for each injection is represented by the horizontal bar. The animal number, date of injection, volume injected, and muscle injected follows each bar. One injection of old, ineffective toxin is not shown. (Repr from *Invest Ophthalmol* 12:925, 1973.)

the range of doses and volume of neurotoxin injected and the observed duration of effect as judged by ocular alignment. An interesting phenomenon was the delay in onset of alignment change and paralysis; in small doses, onset was often delayed 2 to 3 days, with peak effect not seen until 5 to 6 days after injection. This is probably a consequence of the slow release of the toxin into the nerve terminals after binding on the cell surface. Figure 2 demonstrates the change in ocular alignment in the monkey receiving the largest single dose of botulinum neuro-toxin. A left ptosis cleared six weeks following injection. The toxin injected into the left medial rectus produced exotropia which remained stable at 30 prism diopters from three months after injection until transfer of the animal two

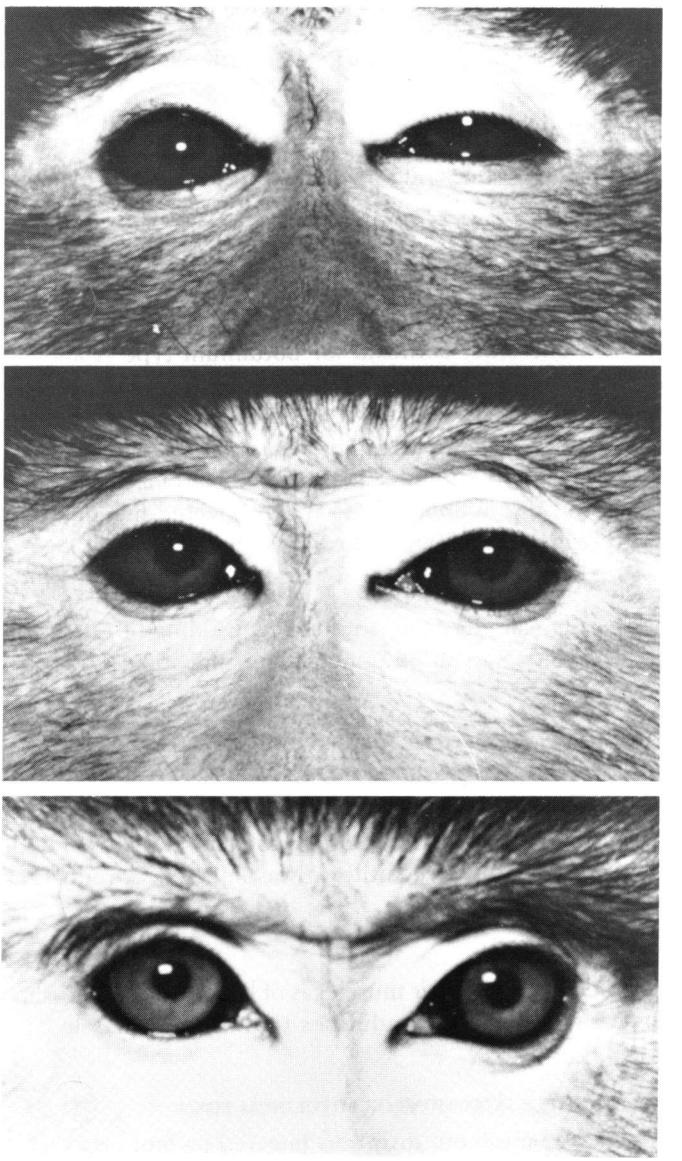

FIGURE 2
Exotropia following injection of left medial rectus muscle. A: Before injection (anesthetized). B: Five months after injection. C: Eight months after injection (animal No 598). (Repr from *Invest Ophthalmol* 12:925, 1973.)

years after injection. Horizontal rotation amplitudes returned to normal, and the EMG recorded from the injected muscle was of normal amplitude.

Conclusion

Drug injection was a safe and reliable method of altering eye alignment in the monkey.

DO LOCAL ANTITOXIN INJECTIONS MODIFY THE EFFECT OF LOCAL TOXIN INJECTION?

Toxin was injected into the medial rectus muscles of both eyes of three monkeys. On one side, antitoxin for botulinum type A and B (Lederle Laboratories) was injected immediately thereafter; on the opposite side saline was injected. There resulted the usual paralysis of the unprotected eye, and almost complete protection from paralytic effects in the injected eye. When injected 30 minutes later, the effect of antitoxin reduced the toxin effect, but did not abolish it. When injected five days later, there was no antitoxin amelioration of toxin effect.

Conclusion

Antitoxin locally can alter the drug effect. Binding of toxin to muscle is probably well underway at 30 minutes after injection.

WOULD INJECTION OF ANTITOXIN PROTECT THE ANTAGONIST MUSCLE (WHICH MIGHT OTHERWISE GET INVOLVED BY DIFFUSION EFFECTS FROM INJECTION OF THE INTENDED AGONIST MUSCLE)?

In two monkeys, toxin was injected into the lateral rectus of both eyes; in the medial rectus of one eye antitoxin was injected. A reduction in paralytic effect in the eye was seen.

Conclusion

Antitoxin injection into other muscles is of limited helpfulness in isolating effect since antitoxin probably diffuses to the target muscle.

EFFECT OF MULTIPLE INJECTIONS OF BOTULINUM TOXIN

In five monkeys botulinum toxin was injected on four occasions, separated several weeks apart. In no instance was a reduction or enhancement of the expected effect seen.

The data from Fiock shows that in humans even large immunizing doses of toxoid in the 15 μg range produce measurable antitoxin titers in only two-thirds of the humans so injected.[25]

Conclusion

Our tiny doses are below the threshold of recognition by the immune system and repeated injections are practical.

SYSTEMIC TOXICITY DOSE IN THE MACAQUE MONKEY

Injection of 10^{-2} μg into monkey 3 produced profound eye muscle paralysis, but insignificant systemic effect, and the monkey survived well.

Injection of 10^{-1} μg of toxin into monkey 610 (3.5 kg) resulted in progressive involvement of the extraocular muscles and the eyelid. The monkey was alive and well three days following injection. Six days following injection the animal was somewhat lethargic and ataxic, and died on the seventh day.

Conclusion

The LD/50 for monkeys probably lies in the region of 0.1 μg. Extension to humans on a weight basis would suggest a human (70 kg) LD/50 dose of about 2.0 μg.

EFFECT OF TOXOID (FORMALIN-TREATED TOXIN)

Could one immunize and protect a patient with toxoid, and then still get a local effect in the injected muscle?

Monkey 597 received pentavalent toxoid 0.5 cc subcutaneously on 10/2/74 and 10/16/74. Two months later, 1.0 μg of botulinum toxin (a very high dose) was injected into the right medial rectus, and no systemic effect of any kind was seen. Two weeks later, 10^{-1} μg were injected into the medial rectus, and no effect of any kind was seen. An additional injection into the medial rectus was made one week later, and again, no effect of any kind was seen.

Conclusion

Botulinum toxoid immunization affords protection against both the systemic and local effect of subsequent botulinum toxin injection.

ELECTRON MICROSCOPY

Eye muscles from four animals were examined through the courtesy of Dr Kazuo Mukuno, of the University of Kitasato, Kanagawa, Japan. Figures here are from the medial rectus of monkey 6, injected with a large dose of toxin (2×10^{-2} μg) one month before sacrifice, at which time muscle function was returning. Sacrifice was by anesthesia and glutaraldehyde

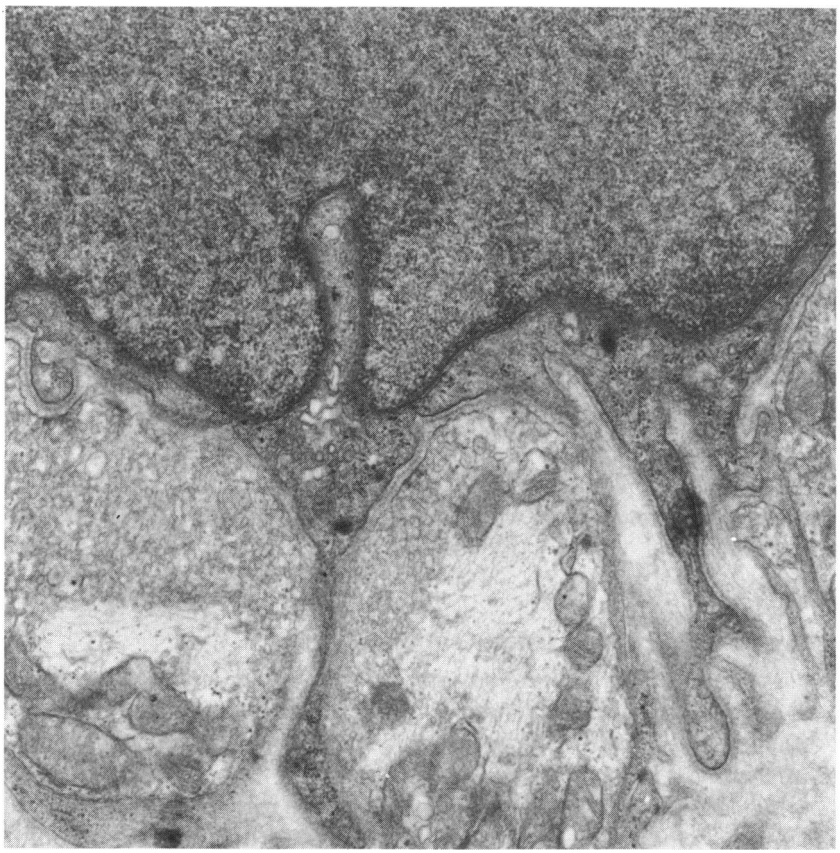

FIGURE 3
Regenerated nerve ending of twitch fiber. Notice the paucity of junctional folds.

perfusion. Nerve terminals of the twitch type showed myelin abnormalities (Figs 3 and 4). Nerve terminals of the slow type are less often abnormal (Fig 5). Muscle fibers appeared nearly normal (Fig 6). In other sections, new nerve sprouts are seen.

Comment

These findings are in keeping with the general notion that toxin effects are reversible, and that function is regained. The greater histologic in-

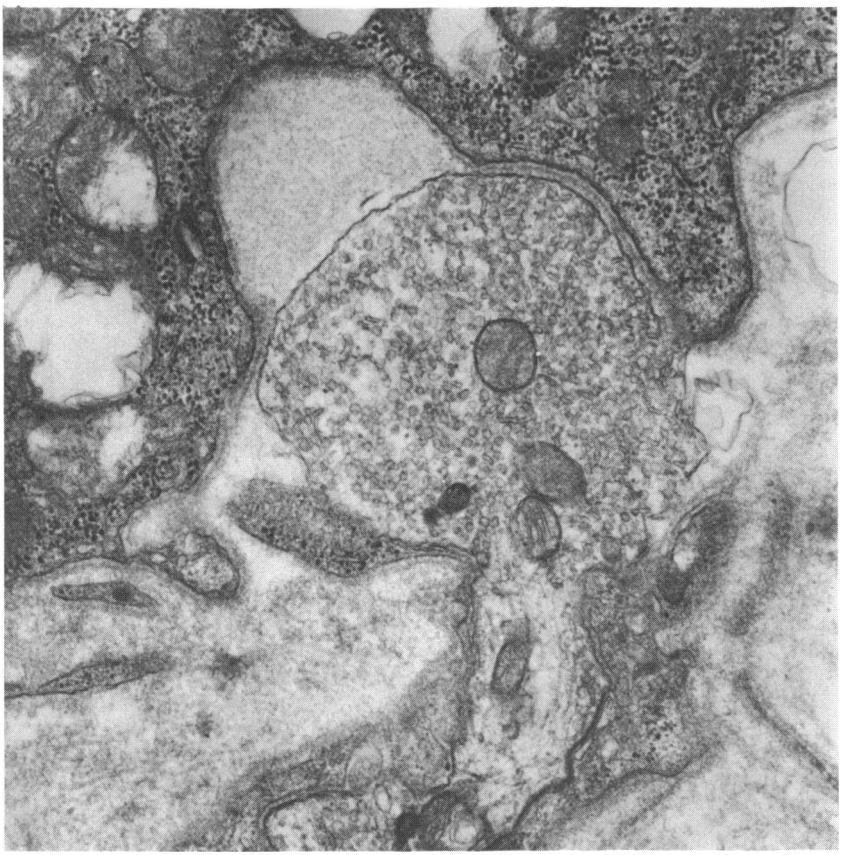

FIGURE 4
Regenerated nerve terminal. Notice homogeneous dense material separating nerve ending from muscle.

volvement on twitch type nerve terminals as compared with slow type nerve terminals is interesting, and possibly important. Drachman[32] examined the effect of toxin on leg muscles of chicks which have a definite population of both slow and fast muscle types, and could not tell a difference in the effect. Duchen and Strich[48] found that as recovery took place, it began sooner and progressed more rapidly in slow muscle than in fast muscle.

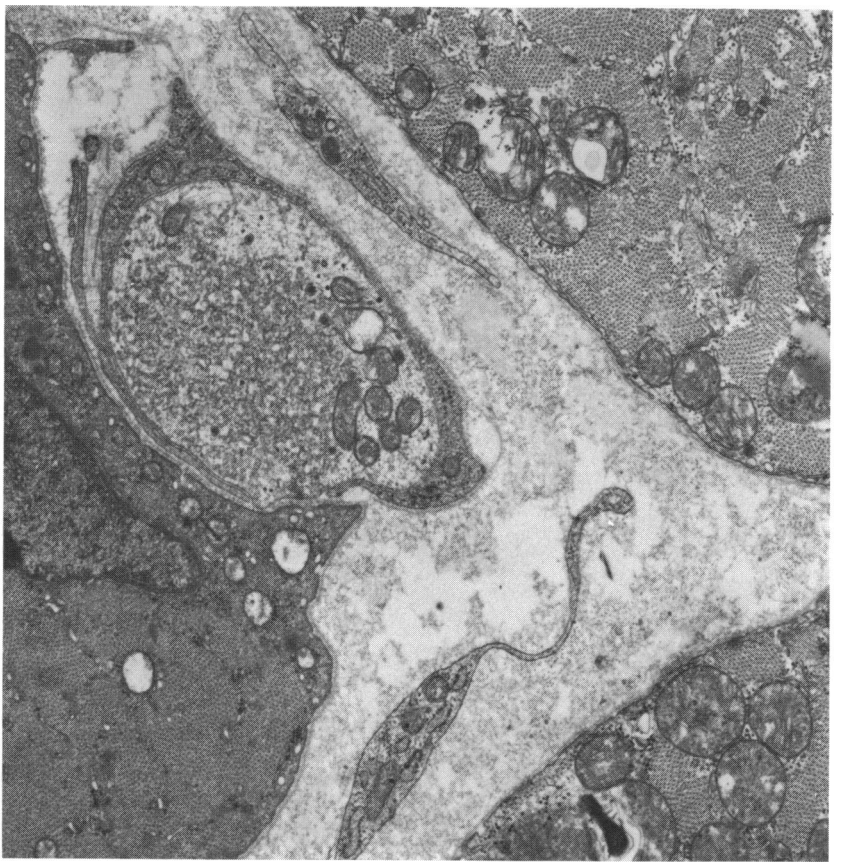

FIGURE 5
A normal-appearing slow nerve terminal.

DIFFUSION OF DRUG WITH INJECTION

In cats, 0.1 cc saline stained with vital blue was injected into extraocular muscles through the conjunctiva. When the injection was made with a single needle tract (no probing and withdrawal of the needle at any time), the injection bolus stayed well localized into the muscle for a period of about 30 minutes. After that time, it began to diffuse through the muscle and outside the muscle itself into adjacent tissues. When the injection

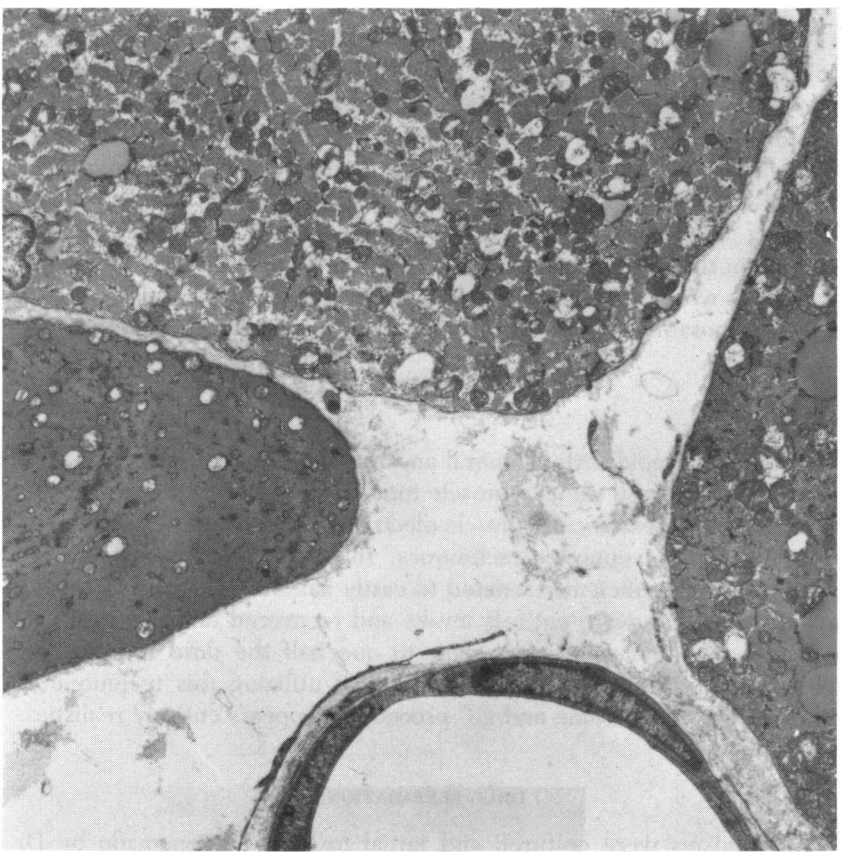

FIGURE 6
Three types of normal-appearing muscle fibers.

was made by thrusting the needle through the muscle and then withdrawing back into the muscle, the injection bolus of 0.1 cc rapidly ran out of the muscle and through the injection tract. From this, it seems quite important to make a carefully planned thrust of the needle into the intended muscle without probing about. With its high blood flow (higher than any but cardiac muscle), it is probable that most toxin which is not bound by the target muscle is flushed away into the general circulation, thereby protecting adjacent muscles.

SIZE OF INJECTION BOLUS

Three monkeys were injected with equal toxin doses, but with volumes of 10, 20, and 100 μl. With smaller volumes, there appeared to be restriction of paralytic effect to the injected muscle with less involvement on the levator muscle or adjacent extraocular muscles. However, in every injection, there was some moderate involvement of adjacent muscles. We concluded that a smaller volume is better. However, a practical difficulty in using very small volumes is the uncertainty of a small air bubble existing in the injection needle itself (volume is about 20 μl). Therefore, in humans we use 50 to 100 μl, which avoids this problem and also allows use of disposable tuberculin syringes rather than special microliter syringes.

ANESTHESIA

Ketamine is a rapid acting general anesthetic producing anesthesia with normal or enhanced skeletal muscle tone. Unlike other anesthetics, this allows sufficient extraocular muscle electrical activity to localize the injection by electromyographic techniques. In small doses (3 to 5 mg/kg), monkeys were sufficiently sedated to easily handle them, and inject the eye muscle; they were entirely awake and recovered in an hour. These doses in monkeys are one quarter to one half the dose required for surgical anesthesia. Thus, the prospect of utilizing this technique in children at a brief "come and go" procedure, appears entirely realistic.

DRUG PREPARATION

The organisms were cultured and initial toxin extraction made by Dr Edward Schantz.[49] The toxin was diluted and freeze-dried in individual vials containing 50 nanograms of botulinum toxin type A, 500 μg of serum albumin, and 900 μg of sodium chloride. Three such lots have been prepared, and used in humans, all of similar characteristics and effectiveness. Sterility, safety, and effectiveness tests have been done under FDA supervision on each lot under our investigational new drug request BB-IND 723.

STABILITY OF THE DRUG

Bioassay is done by injecting four or more mice at each of several dosage levels. Mortality of each group of animals is determined on the third day after injection and the LD/50 calculated from these data. Figure 7 shows a reduction in effectiveness when the drug is stored at room temperature or at refrigerator temperatures after this preparation. However, at freezer

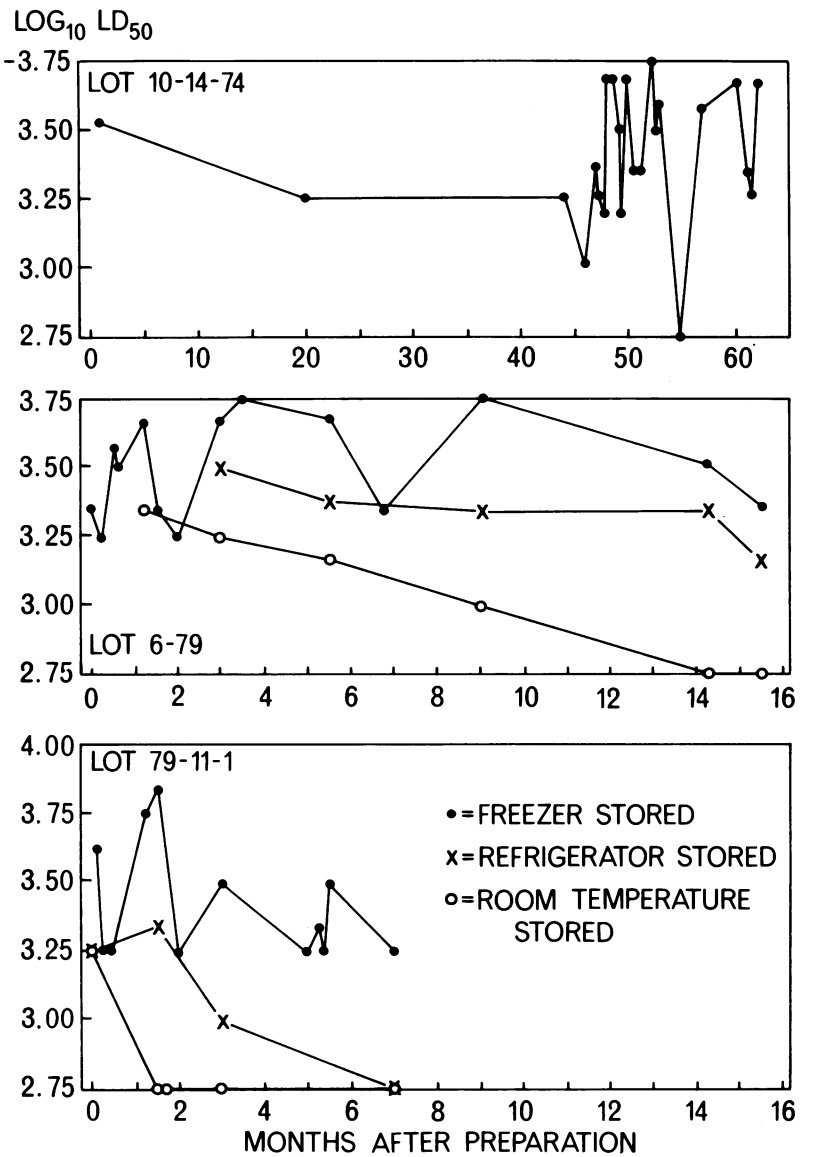

FIGURE 7
Effect of storage temperaure on stability of the drug.

storage temperatures the drug has been stable for up to four years without loss of potency. Since freezer storage is simple, this has been followed. It is readily seen that a few days of storage or exposure to normal temperatures creates no substantial decline in effectiveness, allowing shipping of toxin by ordinary mail or delivery.

SAFETY OF THE TOXIN

This drug has been placed in vials at a level which is 40 times below the estimated lethal human systemic dose. The material rapidly reduces in potency when left in the open or spilled; thus the drug "self destructs." The Communicable Disease Center informed us that no human intoxication has ever resulted from toxin used in the laboratory; none has occurred in our experiments.

EFFECT OF DILUTION ON POTENCY

When reconstituted and allowed to sit covered in the refrigerator for one week, the LD/50 for a gelatin containing solution in mice was between 3×10^{-5} and 1×1^{-4} μg, whereas the same solution mixed without gelatin had an LD/50 of between 1×10^{-4} and 3×10^{-4} μg. However, the effect of the protein additive in the reconstituting solution is not significant when the drug is mixed and injected into patients or animals within one to two hours.

SALINE VERSUS PHOSPHATE BUFFER FOR DILUENT

Phosphate buffer at pH 6.8 is the classical diluent. In a study of 48 mice, there was no difference in the LD/50 for mice injected with buffer solution or plain saline solution of drug mixed and used within one hour. We use saline in humans.

PREPARATION OF HUMAN ANTITOXIN

The author was immunized with toxoid (a formalin inactivated botulinum toxin) at weekly intervals for three doses. Two weeks later, plasma were removed and assayed. One hundred and twelve mouse LD/50 doses of antitoxin per cc were found. There has been no occasion to utilize this human antitoxin. However, the potential for creating a human antitoxin, rather than the present animal-derived antitoxin is quite practical.

INJECTION ELECTRODES

Concentric 26 gauge needles are prepared with a wire down the center in standard bipolar concentric electrode fashion, leaving also a channel for the injection (Fig 8). Building, sharpening, and maintaining such electrodes is a substantial challenge, and I am grateful to Mr Robert Bowen and Mr Lee Tate for expertise and development of these items.

A monopolar needle technique is also possible. This is done by insulating an injection needle except for the tip, and recording signal against a ground wire placed subcutaneously or on the skin. In previously uninjected muscles with a full and strong signal, this is a satisfactory technique and certainly much simpler. Where the EMG signal is not strong (anesthesia, prior injection, etc), the bipolar electrode is helpful.

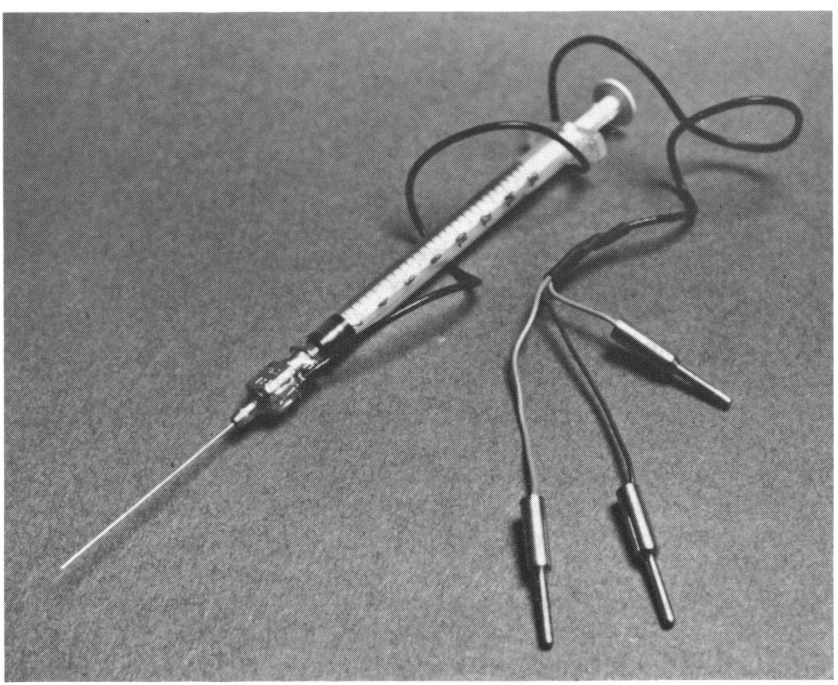

FIGURE 8
Electromyographic-injection needle.

AMPLIFIER DEVELOPMENT

A battery driven differential amplifier has been elaborated by Mr Robert Bowen with the help of Dr Erich Sutter and Mr Al Alden. This allows the project to be moved into the clinic. The audible amplification of the EMG signal is a satisfactory guide to insertion of the electrode. One readily learns to know the sound of insertion potential created as the electrode penetrates membranes, and learns to differentiate the low amplitude distant signal from the high amplitude and crackling sound when the center of the muscle is reached. After many hundreds of injections, I still find extraocular muscles elusive, and difficult to reliably inject without such EMG guidance.

I considered ultrasonography and roentgen ray elaborations (fluoroscopy, CAT scan) as techniques to guide needle placement, but concluded that these cumbersome techniques would add greatly to the cost and time for a procedure which typically lasts about five minutes.

HUMAN EXPERIMENTS

RATIONALE

Consider the evolution of VI nerve paralysis. If the paralysis is mild in extent and brief in duration, recovery can be complete with no residual whatsoever. If the paralysis is more long-lasting, there is often contracture of the medial rectus muscle and stretching of the lateral rectus muscle during the period of paralysis; then although the recovery of the lateral rectus can be quite complete, a nearly concomitant esotropia results. It is just this that we hope to create: controlling intensity of paralysis by varying the injection doses; controlling duration of paralysis by varying the injection dose and by re-injection at intervals. Persistent long-term weakness of extraocular muscles is not the goal: most strabismus is concomitant in nature and non-paralytic concomitant change in deviation is the desired treatment outcome.

To accomplish this, we need drugs which are effective for days to weeks, controllable by dosage variation, devoid of major ocular or systemic side effects, able to be re-injected without allergy or loss of potency from immune response, and which leave the muscle normal after its effects. Botulinum A toxin fulfills these criteria well.

METHODS

CLINICAL MEASUREMENTS

Ocular alignment was assessed by prism-cover test in seeing patients, by corneal reflex in others, and by photography of alignment in all patients. Speed of saccades into the field of action on the involved muscle and active force (isometric) testing were done. The amplitude of rotation into the field of action of the injected muscle was measured in eyes with good vision (Fig 9). In lid retraction, photographic measurement before and after injection is used. Definition of drug effects are in Table III.

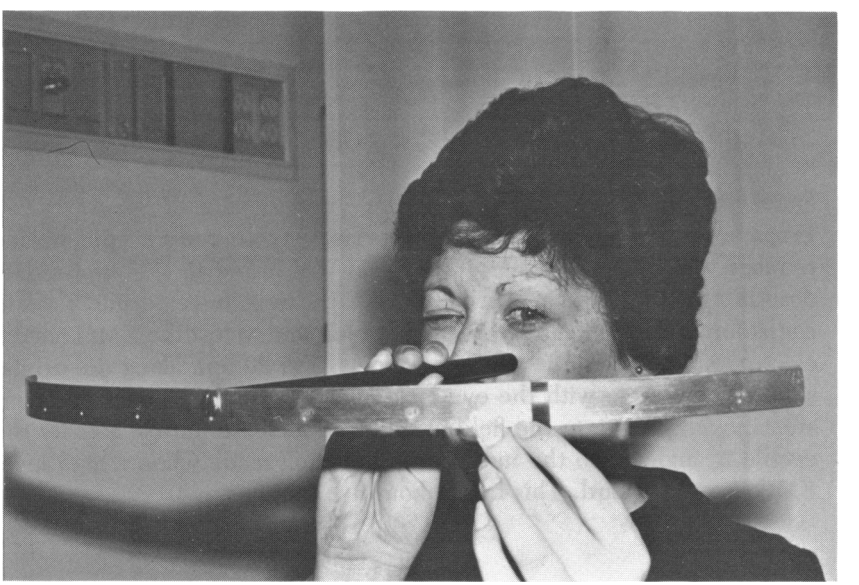

FIGURE 9
Perimeter used to measure duction amplitude.

TABLE III: DEFINITION OF TOXIC EFFECTS

	MILD	MODERATE	MARKED	EXTENDED
Alignment in primary position: change in prism diopters (Δ)	to 10Δ	to 20Δ	to 30Δ	over 30Δ
Rotational amplitude (reduction of baseline amplitude)	−1 (0–20%)	−2, −3 (20–50%)	−3, −4 (50–100%)	−3, −4 (50–100%)
Velocity (Saccades into field of muscle) (% reduction)	to 20%	20–50%	50%	over 50%
Isometric force (from opposite gaze into field of muscle) (% reduction)	to 20%	20–50%	50–100%	70–100%
Duration of effects	to 7 days	to 30 days	to 60 days	over 60 days

INJECTIONS

Proparacaine drops are used for anesthesia. One drop of 1% epinephrine reduces vascularity of tissues. The toxin was mixed to the appropriate dose in a 0.1 cc volume. About 0.3 cc extra were injected into a waste container to clear the needle of any bubbles, and to test the EMG needle electrode. The needle is advanced about 15 to 20 mm along the orbital side of the muscle with the eye looking in the primary position. Then, after moving gaze into the field of action to increase muscle activity the needle is angled into the muscle and injection made when a high level EMG signal is heard. This takes about five minutes.

PATIENT DATA

Table IV lists all patients injected in their order of enrollment in the study. We are interested in all forms of strabismus and make little effort to recruit particular types. All patients had stable deviations for 6 months or more, prior to injection (except lid retraction and acute paralysis). A comparison of injection results and surgical results is planned.

TABLE IV: BOTULINUM TOXIN INJECTIONS

PATIENT	AGE	CONDITION (INITIAL PRISM DIOPTERS)	CONDITION (FINAL PRISM DIOPTERS)	DAYS: LAST INJECTION TO FINAL CONDITION	COMMENT	PRIOR EYE MUSCLE OPERATIONS	DOSES (μg) AND EFFECTS: 0.0000125 OR LESS	0.0000126 TO 0.0000625	0.0000626 TO 0.000312	0.000313 TO 0.000156	0.00157 to TO 0.0078
1	26	20 LXT	20 LXT	30	Discontinued treatment		None None				
2	75	12 RET	12 RET	30	Discontinued treatment		None None				
3	26	a) 35 ET b) 10 RH	25 ET -O-	233	a) Lateral rectus paralysis b) Hypertropia	5	None None				
4	30	25 LXT	10 LET	35	Neuromyotonia lateral rectus (after brain tumor)	1	None None None	Mild	Moderate Moderate	Marked Moderate Moderate Marked	
5	43	35 LXT	±2 X	411	Blind LE	1	None None None	Mild	Moderate Moderate	Marked	Extended
6	24	45 LXT	12 LXT	126	Sickle cell, blind LE	1	None None	Mild	Moderate	Marked Marked	
7	24	16 RXT	12 XT	361	Fusion result	4	None None	Mild	Moderate Marked		

TABLE IV: (CONTINUED)

PATIENT	AGE	CONDITION		DAYS: LAST INJECTION TO FINAL CONDITION	COMMENT	PRIOR EYE MUSCLE OPERATIONS	DOSES (μg) AND EFFECTS				
		INITIAL PRISM DIOPTERS	FINAL				0.0000125 OR LESS	0.0000126 TO 0.0000625	0.0000626 TO 0.000312	0.000313 TO 0.000156	0.00157 to TO 0.0078
8	41	40 LXT	40 LXT	30			None				
9	70	Lid	2 mm drop upper lid	14	Lid retraction, corneal ulcer			Mild	Moderate	Moderate	
10	27	6 LH	10 LH	53		2	None	Mild			
11	38	20 LET	4 ET	427		1	None	Mild	Mild/moderate Moderate Marked		
12	43	16 RET	15 E	20	VI palsy; bilateral injections				Mild Moderate		
13	48	25 RET	14 ET	604	Fusion result	1			Moderate/ marked		
14	33	40 LXT	25 LXT	408	Pre-phthisical	1					Extended
15	39	8 ET R gaze	2-3 ET R gaze	335	Fusion; bilateral injection	2		Mild Mild	Moderate		
16	33	RXT	2 XT	195	Fusion	2		Mild	Moderate/mild		
17	59	60 ET	50 RET	65	Bilateral VI palsy; bilateral injection			Mild	Moderate Moderate Moderate Moderate Moderate		

TABLE IV: (CONTINUED)

PATIENT	AGE	CONDITION		DAYS: LAST INJECTION TO FINAL CONDITION	COMMENT	PRIOR EYE MUSCLE OPERATIONS	DOSES (μg) AND EFFECTS				
		INITIAL PRISM DIOPTERS	FINAL DIOPTERS				0.0000125 OR LESS	0.0000126 TO 0.0000625	0.0000626 TO 0.000312	0.000313 TO 0.000156	0.00157 to TO 0.0078
18	19	25 RXT	16-18 XT	434		1			Moderate Moderate		
19	35	25 RXT	20 XT	180	Fusion; drug effect unusually little	3			None None None None Mild	Moderate	Marked
20	30	25 RXT	±5 X	200	Cerebral palsy	1			Moderate Moderate Moderate		
21	35	Lid	1 RHO	305	Lid retraction; effect unusually great, with some LSR palsy for 3-4 months				Extended		
22	25	20-25 LXT	2 X	210	Fusion result	1			Moderate Moderate		
23	15	45 XT	±25 XT	160		1			None Mild	Mild Moderate	Marked
24	51	25 LXT	10 LXT	42	Medial rectus paralyzed by injury; lateral injected during acute phase				Marked		

TABLE IV: (CONTINUED)

PATIENT	AGE	CONDITION (PRISM DIOPTERS) INITIAL	CONDITION (PRISM DIOPTERS) FINAL	DAYS: LAST INJECTION TO FINAL CONDITION	COMMENT	PRIOR EYE MUSCLE OPERATIONS	DOSES (μg) AND EFFECTS 0.0000125 OR LESS	0.0000126 TO 0.0000625	0.0000626 TO 0.000312	0.000313 TO 0.000156	0.00157 to TO 0.0078
25	17	30 RXT	30 XT		Cerebral palsy; injections not possible under topical anesthesia	1		Mild	Mild		
26	37	25 RET	4 RXT	92	Fusion; transient RSO paresis from RMR injection	1			Moderate	Moderate/ marked	
27	20	25 XT	-O-	112					Mild	Moderate	
28	35	15 LHO	10 LHO	157		2			Moderate		
29	34	30 ET	16 ET	158		1			Marked	Marked Moderate	
30	80	40 RXT	22 RXT	128	Oldest in series				Mild	Moderate	Marked
31	76	25 RHO	20 RHO	110	Endocrine hypotropia, little effect			Mild	Mild	Mild	
32	12	40 LET	-O-	82	Youngest in series					Moderate Marked	
33	16	20 LET	8 ET	74		1			Mild* Moderate		
34	26	35-40 LXT	8 XT	84		1			Moderate		

TABLE IV: (CONTINUED)

PATIENT	AGE	CONDITION (PRISM DIOPTERS) INITIAL	CONDITION (PRISM DIOPTERS) FINAL	DAYS: LAST INJECTION TO FINAL CONDITION	COMMENT	PRIOR EYE MUSCLE OPERATIONS	DOSES (μg) AND EFFECTS 0.0000125 OR LESS	0.0000126 TO 0.0000625	0.0000626 TO 0.000312	0.000313 TO 0.000156	0.00157 to TO 0.0078
35	25	35-40 LET	20 XT	43		2			Moderate Marked		
36	51	25 LXT	16 ET	42		5			Moderate	Moderate	
37	55	Lid	2-3 mm drop lid	7					Moderate Moderate		
38	60	20 RET	-O-	61	Fusion	1			Moderate		
39	24	30-35 RET	20 XT, 10 RH	54	Large effect, hypertropia from injection RMR	3				Extended	
40	20	35 RET	4 ET	46	Fusion					Marked	
41	26	20-30 LXT	5 ET	19	Uveitis, glaucoma, NLP					Marked	
42	33	20 LET		5		3				Marked	

NOTE: The effect is graded according to Table III. The average mouse LD/50 was 4.36×10^{-4} μg. Two injections not listed were external to the muscle rather than into it—no effect resulted. Also not included are five ineffective injections done in April 1979 with an improperly prepared vial. Three of the injections were simultaneous injections of both medial rectus muscles.

RESULTS AND DISCUSSION

The threshold effect for any amount of paralysis lies between 1.25×10^{-5} μg and 6.25×10^{-5} μg. Seven patients received both these doses and all responded uniformly. The effect in all patients can be seen in Table IV. The more the dose the greater and better maintained the response, Figures 11, 12, 13, 14, and 15 show the effect and evolution of treatment in several conditions. With the exception of the small doses used early in the series, for paralytic strabismus and lid retraction, all cases have been helped. The seven cases followed for over one year are maintaining their deviation at the desired level and they are either symptomatically or cosmetically comfortable, and do not wish further treatment (Fig 10).

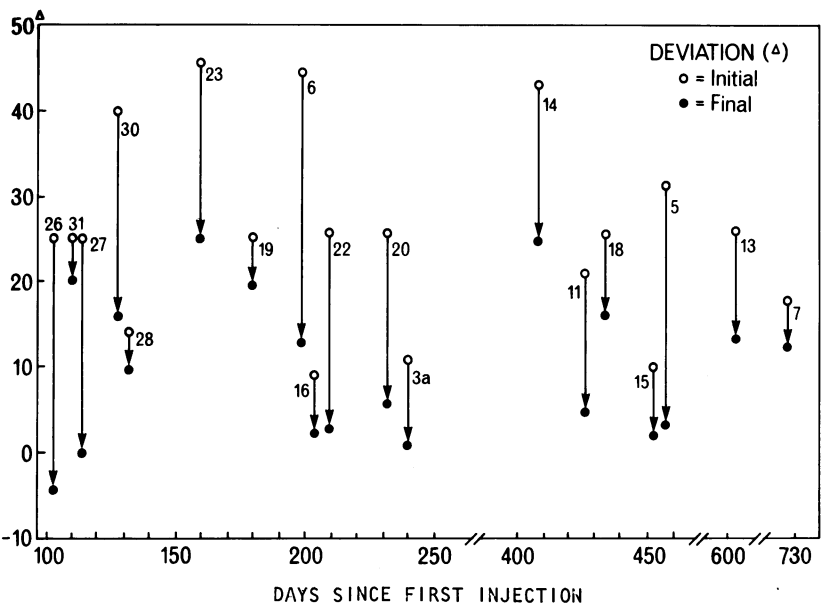

FIGURE 10

Injection effects. Excluded are: Doses less than 3.12×10^{-4}, the therapeutic threshold (1, 2, 8, and 10); paralytic strabismus (3a, 12, 17, and 24); eyelid injections (9, 21, and 37); followed less than 100 days since last injection; neuromyotonia (4).

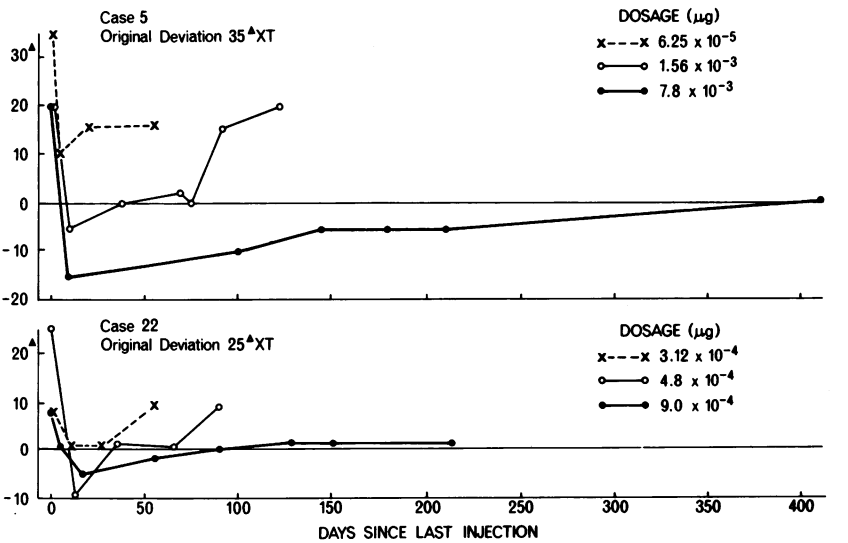

FIGURE 11
Change in deviation (ordinate) with successively larger doses of botulinum A toxin.

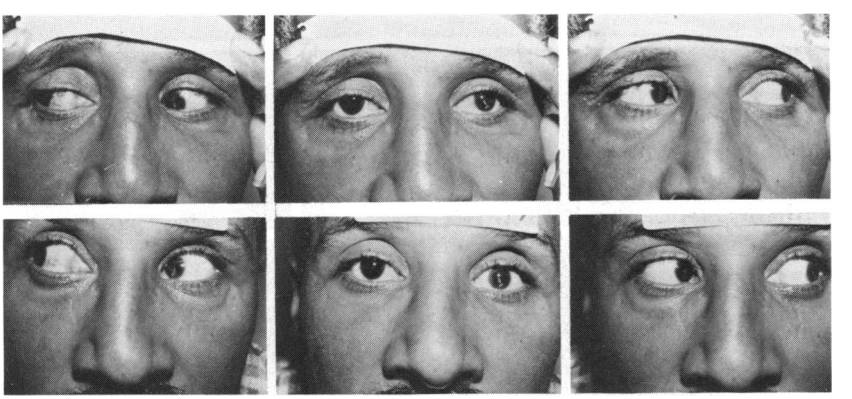

FIGURE 12
Patient 5. Old injury to left eye with secondary exotropia. Top: Before injection of 2.5×10^{-6} μg. Bottom: Twenty-six days after injection of 3.125×10^{-4} μg into left lateral rectus.

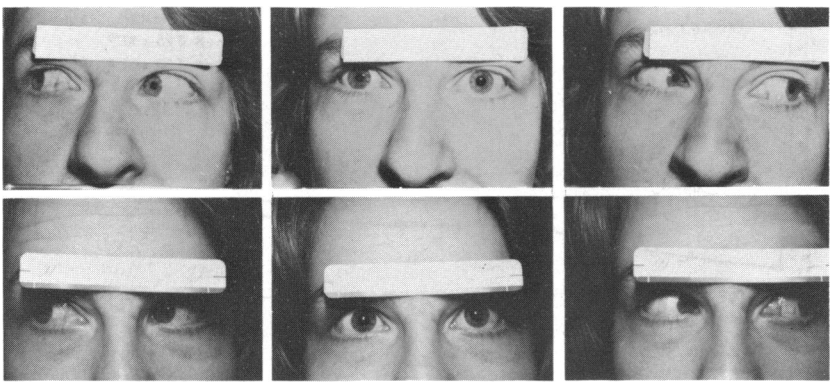

FIGURE 13
Patient 7. Residual exotropia after surgery four years earlier. Top: Before injection. Bottom: Thirty days after injection of 3.125×10^{-4} μg into right lateral rectus. Notice moderate abduction weakness and right esotropia.

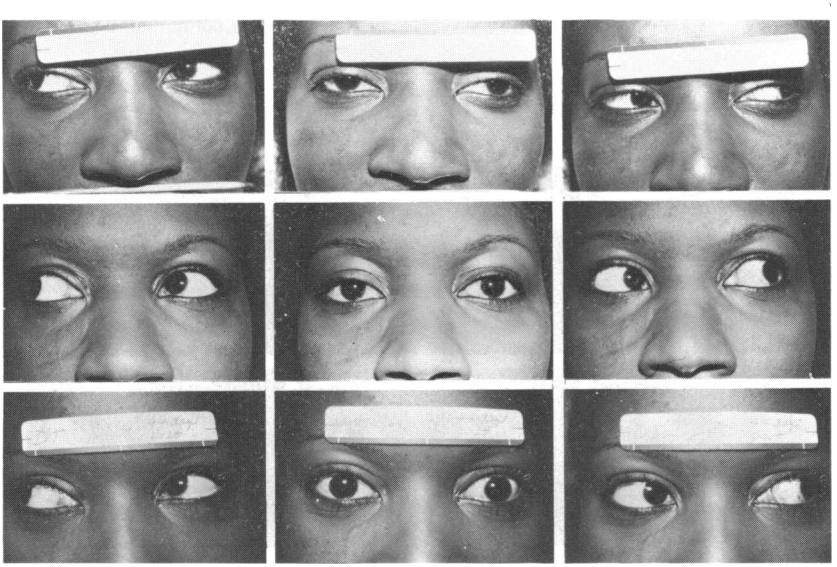

FIGURE 14
Patient 6. Blind left eye from retinal detachment due to sickle cell disease. Top: Before injection. Middle: Twelve days after injection of 3.125×10^{-4} μg. Bottom: Forty-four days after injection of 3.125×10^{-4} μg into left lateral rectus. The effect is declining at 44 days.

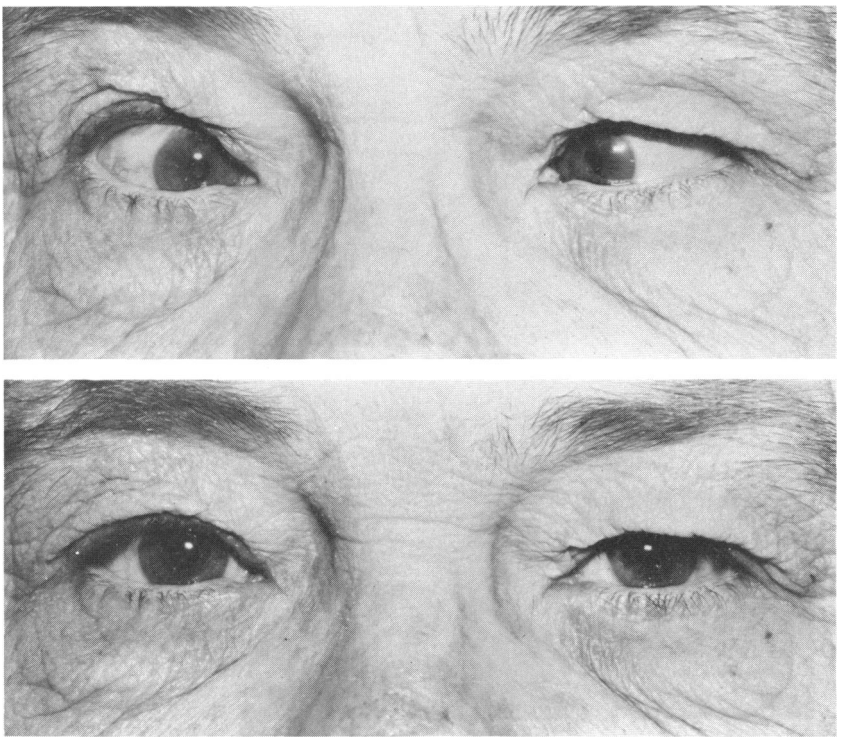

FIGURE 15
Patient 17. Bilateral VI nerve paralysis. Top: Two months after head injury. Bottom: Twenty-one days later, after drug injection of medial rectus muscles.

MULTIPLE INJECTIONS

No patient has shown any tendency to tolerance. For example, patient 3 received nine injections in the horizontal muscles in an attempt to correct his paralytic strabismus (with only limited success). Thereafter, two injections into the superior rectus to correct a vertical deviation were normally effective in creating paralysis and developing a long-term alignment change.

VARIATION IN RESPONSE

There were exceptional responders among our subjects. Patient 21 was injected (levator) on the same day with the same dose from the same vial

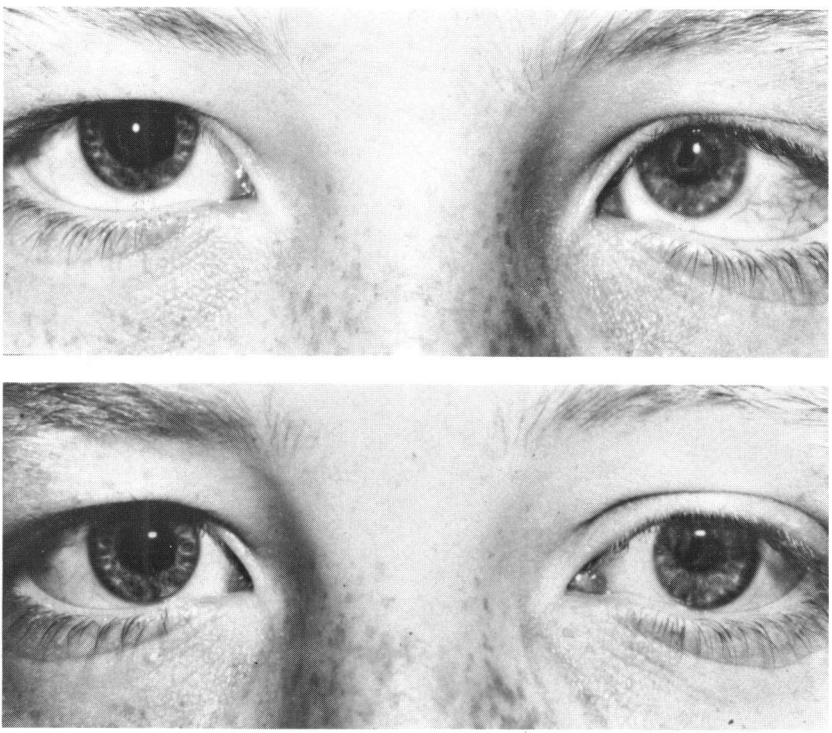

FIGURE 16
Patient 32. Age 12 (youngest subject). Top: Forty prism diopters LET. Bottom: Eighty-two days since last injection, 1.56×10^{-3} μg.

as patient 19 (lateral rectus). The effect in patient 21 was a marked weakness of the injected muscle over an extended period of time. Patient 19 got only a mild response which lasted not over 7 to 10 days, and later required a dose ten times larger. No other extraordinary responses approaching these extremes, but I tend to make the initial injection at a moderate level for the condition existing, with the recognition that the majority of patients will require a second injection at a later time. Since a prolonged period of weakness is advantageous, this second injection carries with it some positive value. Ultimately, I estimate that about 40% of patients will be adequately treated by a single injection, perhaps another 40% by the second injection, and further injections will be required as part of the initial treatment series for the remainder.

There is a marked variation of toxin effectiveness in various species, and in human botulism epidemics a tremendous variability exists. The basis for these differences is not known. Our two most sensitive eye muscles were in young women; the most unresponsive muscles were in husky and tall men. Our youngest patient, age 12, weighed 100 lbs and showed a response equal to adults.

INJECTION WHERE STRABISMUS SURGERY IS NOT INDICATED

Anterior segment ischemia from a retinal detachment procedure was present in patient 38. Strabismus surgery on the unoperated horizontal muscle was not considered safe. Injection was spectacularly helpful in aligning his eyes, regaining fusion and removing his diplopia. Patient 40 had an intraocular pressure of less than six in a blind eye with an existing retinal detachment and 60 prism diopters of exotropia. He was advised by a glaucoma consultant that a 50% chance of phthisis existed if strabismus surgery were done. He elected an injection which has placed his eye in a satisfactory alignment position. Patient 41 had an intraocular pressure of 60 mm/Hg with no light perception and continuing uveitis in an eye previously operated for cataract. These and similar situations represent an area where the technique fulfills a need without supplanting existing modalities of treatment. Table IV shows that most of these patients had been operated upon, had a residual strabismus, and sought alternative treatment.

BILATERAL INJECTION

This has been done in three cases without systemic or other complication, and should carry the same indications as bilateral strabismus surgery. For example, bilateral medial rectus weakening in high AC/A ratios (patient 15).

COMPLICATIONS

The following complications or adverse effects have been encountered.

1. Corneal irritation. Drying of the cornea or physical irritation must be carefully avoided.
2. Sub-conjunctival hemorrhage from needle insertion. This is unusual with the point-sharpened (no knife edges) needles. A drop of epinephrine to the conjunctiva to shrink conjunctival vessels makes them less likely to bleed. It also increases visibility of the deep anterior ciliary vessels to allow their avoidance. Vertical rectus muscles are especially prone to such hemorrhage because of the larger number of anterior ciliary arteries associated with them.

3. Discomfort at time of injection. This usually indicates inadequate topical anesthesia. Five drops of proparacaine over a five minute period is a minimum. Injection of a small amount of local anesthetic anteriorly is acceptable but usually unnecessary.
4. Prolonged discomfort lasting a few hours. This is usually associated with a needle touching the orbital periosteum or encountering scar tissue from a prior operation. There is no really good treatment except avoidance and analgesic medication.
5. Effect of the drug on adjacent muscles. In doses greater than 1.56×10^{-3} μg this is seen almost uniformly. The levator appears to be especially susceptible. These effects on adjacent muscles typically occur only when the injected muscle itself receives a strongly weakening effect. In every instance, but one, it has disappeared completely. When surgery was done three months later in that case for the persistent vertical deviation, the induced horizontal deviation was also corrected.
6. Injection not into the muscle. On one or two occasions injection was not directly into the muscle. One can expect insufficient or no effects under these circumstances.
7. Disorientation of the patient. Past-pointing and spatial disorientation occur with paralysis of a fixing eye. When this is a problem, that eye should be covered. Adaptation to the paralysis typically occurs after the third or fourth day.
8. Diplopia. Frequently an eye is turned by the injection temporarily to a position where no suppression occurs. Occlusion of the eye is indicated.
9. One patient developed a slight rash a day or two following injection. The attending physician thought this to be a neurodermatitis rather than an allergic reaction. It cleared spontaneously.

COMPLICATIONS NOT ENCOUNTERED

1. Penetration of the sclera. With visual guidance of the needle tip sub-conjunctivally and exterior to the muscle to a position at least posterior to the equator, and EMG guidance of the needle thereafter, this complication should never occur.
2. Serious retrobulbar hemorrhage. Since the needle should be exterior to the muscle cone or within the muscle, and never within the muscle cone, this should never occur.

3. Pupilo-motor and accommodation changes. These have not been encountered. The combination of proparacaine drops and epinephrine drops does cause some pupil enlargement at the time of injection in most patients.
4. Systemic effect. No paralytic effect has been seen or suspected in any patient.

LEVATOR

Injection of the levator in lid retraction was done in patients 9, 21, and 37 with only mild long-term effect. This is probably due to the relative inactivity of the orbicularis muscle during most of the day—this antagonist muscle is not continuously innervated as are the extraocular muscles. Injection of the upper lid as a temporary technique to close the lid for corneal exposure works nicely, with good recovery subsequently. This is therapeutically more attractive than tarsorrhaphy techniques which are generally applied, and should be a major area of utility.

VERTICAL MUSCLES

It is a practical problem to restrain effect to an individual vertical muscle without effect on adjacent muscles. In patient 31, (inferior rectus) and in patient 21 (levator) significant effect on adjacent muscles occurred lasting over a month. However, in patient 10, (inferior rectus) patient 28, (inferior rectus) and patient 9, (levator) no effect on adjacent muscles has occurred, and therapeutic levels of effect have occurred in the injected muscle. Thus, particular care and restriction of dosage size to 0.05 ml may be important in injection of these muscle areas.

PARALYTIC STRABISMUS

Injection of the medial rectus in recent lateral rectus paralysis was spectacularly successful in bringing the eye back toward the primary position and in preventing contracture (patient 17, Fig 14). This approach was used to prevent contracture of the antagonist when a muscle had been damaged (patient 24). However, long-term effect does not occur. Without the antagonist to take up the slack created by paralysis, the eye simply drifts back to the original position (patient 3a and patient 12). Thus, the toxin should not be used as a treatment for chronic paralytic strabismus cases. The effect in moderate vertical palsy, especially superior oblique palsy, has not yet been adequately documented. From many EMG studies, the author is aware that few so-called superior oblique palsies are total. Furthermore, there is frequently the presence of other vertical

muscles which might effectively take up the slack from injection of an over-acting antagonist such as the inferior oblique. This question is to be tested.

FUNCTIONAL RESULTS

Unexpected binocularity and fusion has occurred with surprising frequency. In patient 40 there was a history of esotropia since infancy, and an existing deviation of approximately 55 prism diopters without glasses (25 prism diopters with hyperopic correction). Definite motor fusion was regained. Similar fusion has developed in patients 11, 13, and 29. I speculate that the marked incomitancy which exists during the period of paralysis allows the eyes to be exotropic in one gaze position, esotropic in the opposite gaze position, but usually there is some point where the two eyes are perfectly aligned. Thus during all waking hours constant stimulation of the retina on both sides of the fovea, plus the opportunity to tune the two foveas together by small head movements, apparently provides the opportunity to develop or to regain binocular fusion to a degree not obtainable in the usual surgical correction.

SUMMARY

One hundred thirty-two doses of botulinum A toxin were injected into 42 humans. The effect on horizontal strabismus was uniformly beneficial, and effect lasting up to 411 days since the last injection was documented. The effect in vertical strabismus and lid retraction was beneficial, but less strongly so. No systemic effect or local complications were encountered except for effect on adjacent muscles. The drug appears to be a safe and useful therapy for strabismus.

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